J. HYM. RES.
Vol. 8(2), 1999, pp. 238–250

Does the Mating System of *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) Allow Outbreeding?

A. D. LOCH AND G. H. WALTER

(ADL and GHW) Department of Zoology and Entomology, The University of Queensland, Brisbane, Queensland, 4072, Australia; (ADL Present address: CSIRO Entomology, c/- Department of Conservation and Land Management, Brain St, Manjimup, Western Australia, 6258, Australia)

Abstract.—The quasi-gregarious egg parasitoid *Trissolcus basalis* (Wollaston) is generally considered to be an entirely inbreeding species because it is a sib-mating species that has female-biased sex ratios. Whether the species also outbreeds has not been previously investigated although several aspects of its mating behaviour suggest this might be possible. This question was investigated indirectly in two ways by quantifying: (1) the inseminative capacity of *T. basalis* males in relation to the rate of female emergence, and (2) the effects of age and mating status on sexual receptivity of *T. basalis*. *Trissolcus basalis* females emerged over a period of several days, concentrating their emergence in the morning hours. Males were able to inseminate many females (> 50) in rapid succession, apparently without sperm depletion. However, approximately 20% of females did not produce female offspring, probably because they did not mate. Although the mated females produced proportionately more male offspring with time, this outcome is not readily explained by sperm depletion of their mating partners and remains an unresolved issue. Male sexual receptivity appears to be unaffected by age and would be expected to be unaffected by mating status because males typically are polygynous. Although a previously successful mating encounter did not preclude females from mating again, female sexual receptivity decreased significantly after mating. Female sexual receptivity also decreased significantly with age. These results suggest that both *T. basalis* males and females have the ability to mate away from the natal site and that outbreeding is possible in this species. Whether males and females can locate one another away from their own natal site therefore warrants further investigation.

Arrhenotokous parthenogenesis is the usual means of reproduction in Hymenoptera. Female-biased sex ratios and sib-mating are characteristic of many arrhenotokous species, especially those whose males develop in the vicinity of their female siblings and emerge before them (protandry). This occurs most frequently in gregarious parasitoids, which deposit many eggs per host, and quasi-gregarious ones (van den Assem et al. 1980), which lay one egg per host into hosts that are invariably aggregated. Hamilton's (1967) local mate competition (LMC) theory is generally seen as the best explanation of female-biased sex ratios (e.g. Waage and Lane 1984; Waage and Ng 1984; Hardy et al. 1993; Godfray 1994; but see Walter and Clarke 1992; Ode et al. 1997). Because female Hymenoptera can control the fertilisation of each egg they deposit, LMC theory predicts that single foundress broods of gregarious and quasi-gregarious species will contain only enough male offspring to mate all of their sisters in the immediate vicinity (Hardy et al. 1998).

Several recent studies have shown that strict local mating does not occur in some species with female-biased sex ratios and which therefore should be inbreeding species (e.g. Myint and Walter 1990; Nadel and Luck 1992; Molbo and Parker 1996; Hardy et al. 1999). The term partial local mate competition has been often used for

such cases. Another species whose mating behaviour appears not to be strictly local is *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), a quasi-gregarious egg parasitoid of the green vegetable bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae). *Trissolcus basalis* is regarded as an inbreeding species that manifests local mate competition (LMC) (Hamilton 1967) because it has female-biased sex ratios and sib-mating (Noble 1937; Anon. 1939; Smith 1945; Wilson 1961; Thomas 1972). However, several aspects of its mating system are inconsistent with LMC and indicate that a proportion of each brood may outbreed if they are to transmit genes beyond the next generation. Field observations indicate that nearly 20% of newly-emerged females depart the egg mass unmated, approximately 25% of mated females were mated more than once and often by multiple males, virgin and mated females remained nearby the egg mass for up to several hours after emergence, and males dispersed from the natal site (A. D. Loch and G. H. Walter unpublished data). Given that both males and females leave the natal site in the field, it is certainly possible that unrelated males and females meet and mate away from their natal site.

The female mating pattern in *T. basalis* may be a consequence of the males mating many females in quick succession, and becoming sperm depleted. Females mated by sperm depleted males may receive insufficient sperm to produce the usual proportion of daughters in a brood. They may therefore mate again, with outbreeding being more likely in such circumstances. The inseminative capacity of *T. basalis* males was therefore investigated in relation to the rate and sequence of female emergence from the host patch. The experiment was designed to emulate typical rates of sibling emergence and mating in the field, rather than the unnaturally high rates used in some studies (e.g. Nadel and Luck 1985). Specifically, the number (and proportion) of females emerging from a single egg mass and inseminated by one male was quantified. The consequence of female emergence position for the amount of sperm received from the male was quantified by recording the number and sex ratio of progeny from every tenth female to emerge. Whether *T. basalis* males become sperm depleted at mating rates typical in the field could thus be determined.

The potential for outbreeding in *T. basalis* was also evaluated by determining whether males and females are sexually receptive after mating and/or leaving the natal site. We therefore investigated the effects of age and mating status (virgin or mated) on *T. basalis* sexual receptivity in the laboratory, by exposing different aged virgin and once-mated females to newly-emerged virgin males. For completeness, the effect of age on male sexual receptivity was also studied by mating different aged virgin males with newly-emerged virgin females.

MATERIALS AND METHODS

Laboratory cultures.—Green vegetable bugs were reared at 28 ± 1°C, 65 ± 10% R.H., 16L:8D in mesh cages (0.45 m sides) on a diet of green bean pods (*Phaseolus vulgaris* L.), shelled peanuts (*Arachis hypogea* L.) and water. Cultures were augmented regularly with field-collected bugs. Green vegetable bug egg masses were collected daily from cages and were used to maintain cultures of green vegetable bug or *T. basalis* (see below).

Laboratory cultures of *T. basalis* were established from parasitoids that emerged from green vegetable bug egg masses collected from mungbean, *Vigna radiata* (L.) Wilezek, and soybean, *Glycine max* (L.) Merr., during March–April 1997 and January–April 1998 at Pittsworth (27° 43′S, 151° 38′E), Bongeen (27° 34′S, 151° 27′E) and Cecil Plains (27° 32′S, 151° 12′E) in south-eastern Queensland, Australia. All *T. basalis* individuals that emerged from a single egg mass were held together in a

ventilated vial streaked with honey. Cultures of *T. basalis* were kept at 15 ± 1°C, 65 ± 10% R.H. and 16L:8D. The identification of *T. basalis* was confirmed by Dr Norman Johnson (Ohio State University). Voucher specimens from the *T. basalis* culture are deposited in The University of Queensland Insect Collection.

In all experiments, virgin wasps of the F_1– F_3 generation were used. Wasp virginity was ensured by holding single wasp pupae in ventilated vials with honey, after breaking the host egg mass into individual eggs soon after parasitoid pupation. For each experimental replicate, wasps were derived from different field-collected egg masses to ensure siblings were not included as replicates.

Inseminative capacity.—To determine male inseminative capacity, all of the *T. basalis* females that emerged from each of 10 parasitised green vegetable bug egg masses were tested for insemination (see below). These original egg masses contained 85 ± 5 eggs, the mean size for green vegetable bug egg masses in southeastern Queensland. Each egg mass had been parasitised by a single, once-mated female *T. basalis* over two days in a 50 × 25 mm ventilated vial. Self-superparasitism is unlikely to arise under such conditions because females use a chemical marker to mark parasitised eggs (Wilson 1961; Ganesalingam 1966; Field et al. 1998). After 9–10 days, when the first males began to emerge, vials containing the parasitised egg masses were monitored frequently (every 5–10 minutes) during the 10 hours of artificial laboratory light each day. Before females began to emerge, all males were removed except for the dominant male occupying the egg mass. The dominant male was lightly marked on the thorax with fluorescent dust to distinguish him from males that emerged subsequently. These latter males were removed immediately they appeared.

At each monitoring period any females that had emerged were removed and each was placed alone in a ventilated vial and provided with honey. Females were typically found at the top of the vial. All females, except those used to assess fecundity (see below), were provided 5–10 fresh green vegetable bug eggs to establish whether they produced female offspring, a certain indication they had been inseminated (Wilson 1961). Females were allowed 24 hours to parasitise eggs before being removed.

Lifetime fecundity was assessed for the first emerging female and for every tenth female that emerged from each egg mass. Each was provided with a frozen (−70°C) green vegetable bug egg mass each day for the first 12 days. The frozen eggs were < 1 month old and still viable for *T. basalis* (Powell and Shepard 1982, Kelly 1987). Earlier trials (n = 6) indicated that daily fecundity decreased rapidly and females were unlikely to produce offspring after 12 days. Large egg masses (85 ± 5 eggs each) were supplied on each of the first two days, half masses (40 ± 5 eggs) for each of the next four days, and small masses (20 ± 5 eggs) for each of the last six days, so that females had an excess of hosts at all times (see Results). Parasitised egg masses were placed singly in ventilated vials and incubated until all offspring had emerged. Eggs that were obviously parasitised, but from which parasitoids failed to emerge, were dissected and the parasitoid removed for sexing. Counts of the numbers of male and female offspring produced per female per day were then made.

Adult size.—Adults were measured to assess whether their size was affected by emergence sequence and whether fecundity was influenced by size. Two measurements were taken from all males and females to emerge from each egg mass: head width and right hind tibial length. Head width was measured as the distance between the outermost points of the eyes.

Measurements were made under a dissecting microscope, accurate to 0.01 mm.

Sexual receptivity.—Two experiments were conducted to investigate the effect of adult age and mating status (virgin or mated) on sexual receptivity. The first examined whether age affected the male's readiness to mate. A single virgin male aged 1, 5, 10, 15 or 20 days old was introduced into one end of a 50 × 12 mm ventilated vial containing a virgin female less than 24 hours old at the other end. The male and female were observed until mating occurred or for 20 minutes, as virgin males and females would usually mate within 10 minutes with an average premating time of *ca* 3 minutes.

The number of contacts between the male and female before mating was recorded, as were the pre-mating and mating times. In addition, pre-mating and mating behaviours were observed for any differences between treatments. Once mating had taken place, the male was removed and the female provided with *ca* 10 green vegetable bug eggs. The eggs were removed one day later and incubated at 28 ± 1°C until offspring emerged. Because *T. basalis* is arrhenotokous (Wilson 1961), a female was regarded as successfully inseminated if any female offspring were produced.

The other experiment examined whether female age and mating status affected her readiness to mate. Virgin females were assigned to two groups. Those in one group were not mated, whereas the others were mated within 24 hours of emergence by a virgin male. All females were held, until needed, in a ventilated vial streaked with honey. Subgroups of females (virgin or once-mated) were exposed to virgin males less than 24 hours old, at ages 1, 5, 10, 15 or 20 days, one pair per 50 × 12 mm ventilated vial. Procedures and conditions were the same as for the first experiment. For both experiments 15 replicates of each treatment were conducted, all at 25 ± 2°C and 65 ± 10% R.H.

Statistical analysis.—Logistic analyses were conducted to test whether emergence position influences the probability of a female being inseminated. A logistic regression was conducted for each of the 10 experimental replicates, in which the binary response variable, whether a female was inseminated (assigned 1) or uninseminated (assigned 0), was regressed against her position in the emergence sequence.

The fecundity of females in different emergence positions and their offspring's sex ratio were analysed by 1-way ANOVA after $\log(x + 0.5)$ and $\arcsin(\sqrt{p})$ transformations, respectively. The significance of any differences was assessed by Fisher's protected least significant difference test. Linear regression was employed to assess the relationship between fecundity and female head width or hind tibial length.

The effect of age of males, virgin females and mated females on the number of pre-mating contacts, pre-mating time and mating time was tested by 1-way ANOVA after data were $\log(x + 0.5)$ trans-

Table 1. Summary statistics from logistic analyses testing whether emergence position influences the probability of a female being inseminated. A logistic regression was conducted for each of the 10 experimental replicates, in which the binary response variable, whether a female was inseminated (assigned 1) or uninseminated (assigned 0), was regressed against her position in the emergence sequence. Relationship refers to whether females later in the emergence sequence tended to be uninseminated (negative) or inseminated (positive).

Replicate	No. females	χ^2	p-value	Relationship
1	78	0.58	0.45	negative
2	40	1.52	0.22	positive
3	30	0.56	0.45	negative
4	73	0.30	0.58	positive
5	59	5.74	0.02	negative
6	55	0.80	0.37	positive
7	60	11.41	<0.01	negative
8	57	0.15	0.70	negative
9	61	2.78	0.10	positive
10	78	2.56	0.11	positive

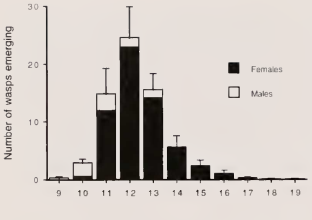


Fig. 1. Pattern in which *Trissolcus basalis* siblings emerged from parasitised green vegetable bug egg masses. Number of males and females that emerged each day from each of 10 egg masses (parasitised on day 0) in the laboratory at 28 ± 1°C, 65 ± 10% R.H. and 16L:8D. Error bars represent standard errors for the mean number of siblings (males and females) that emerged each day.

formed. G-tests were employed to test the effect of age of males, virgin females and mated females on the number of females mated within 20 minutes. G-tests were also employed to test if the number of females inseminated was related to age of males and virgin females. The effect of mated female age on the probability of being inseminated was not analysed statistically because mated females were presumed to have been successfully inseminated at their first mating.

The effects of female age and mating status (virgin or once-mated) on the number of pre-mating contacts, pre-mating time and mating time were tested by two-way ANOVA after data were log(x + 0.5) transformed. A log-linear analysis was employed to test the effects of female age and mating status on the number of females mated within 20 minutes.

RESULTS

Emergence patterns.—Most wasps (> 95%) emerged under lighted conditions, with the majority emerging during the first 3–4 hours of morning light. A mean ± s.e. of 67.9 ± 4.6 wasps emerged from each of the 10 original egg masses, comprising 59.1 ± 5.0 females and 8.8 ± 2.3 males. Males began emerging on day nine with emergence peaking on days 10 and 11 (Fig. 1). Few males, if any, emerged from egg masses later than day 14. Females emerged on days 10–19 with emergence peaking on days 11–13. The largest number of females that emerged from any one egg mass in one day was 57 females on day 12.

Table 2. Overall number of offspring and offspring sex ratio (mean ± s.e.) produced by *Trissolcus basalis* females in different positions in the entire emergence sequence. Fecundity and sex ratio values derive only from those females that had been inseminated (as indicated by their production of daughters). See Fig. 2 and Table 1 for details.

Emergence position	No. mated	No. not mated	Fecundity[1]	Sex ratio (♂ male)[1]
1	8	2	139 ± 13	0.23 ± 0.03
10	8	1	125 ± 17	0.26 ± 0.04
20	9	1	129 ± 16	0.29 ± 0.05
30	9	1	116 ± 11	0.36 ± 0.06
40	8	0	100 ± 12	0.30 ± 0.08
50	7	1	153 ± 23	0.30 ± 0.04
60	5	1	95 ± 15	0.22 ± 0.08
70	1	2	116	0.72

[1] Column means for fecundity ($F_{7,48} = 1.12$, $p = 0.37$) and sex ratio ($F_{7,48} = 1.61$, $p = 0.16$) were not significantly different.

Inseminative capacity and fecundity.—The dominant males that were left alone on egg masses to mate their sisters inseminated a mean ± s.e. of 48.3 ± 3.9 females, with 68 females being the maximum number inseminated by one male. The proportion (mean ± s.e.) of emerging females that was inseminated by the dominant males was 0.82 ± 0.02 across egg masses. Of the 10 replicates conducted, five showed a positive relationship between the probability of a female being inseminated and her emergence position and five showed a negative relationship (Table 1). Two of the negative relationships were significant at < 5% and two of the positive relationships were significant at < 11% (Table 1).

Table 3. Number and sex ratio (mean ± s.e.) of offspring produced by females that emerged as adults during a single day, but in different positions in the emergence sequence. Only females that emerged on the first day of female offspring emergence were included in the analysis because at this stage males would presumably have had a full sperm supply, and short term rates of sperm depletion could be assessed most accurately. Fecundity and sex ratio values are calculated only from mated females in each position.

Emergence position	No. mated	No. not mated	Fecundity	Sex ratio (♂ male)
1	8	2	139 ± 13	0.23 ± 0.03
10	6	0	112 ± 19	0.27 ± 0.04
20	3	1	150 ± 4	0.27 ± 0.05
30	2	0	108 ± 4	0.46 ± 0.17
40	2	0	99 ± 45	0.21 ± 0.00
50	1	0	257	0.35

Column means for fecundity ($F_{5,16} = 2.31$, $p = 0.09$) and sex ratio ($F_{5,16} = 1.47$, $p = 0.25$) were not significantly different.

Fecundity was highly variable, and ranged from 42 to 257 (mean ± s.e. = 121 ± 5, n = 65) offspring per female. Progeny production peaked during the first 24 hours after emergence with about 40–50 offspring on average, and then decreased rapidly with time (Fig. 2). The number of offspring produced per day was always less than the number of hosts provided. The sex ratio (proportion male) of offspring increased with time such that females produced few or no female offspring after 10 days, although by then few offspring were being produced (Fig. 2). Fecundity and brood sex ratio were not significantly affected by the position of parent females in the overall emergence sequence (Table 2), nor by the position of females in the emergence sequence on the first day of female emergence (Table 3).

Adult size.—Head widths and hind tibial lengths for male and female *T. basalis* showed little variation within and across replicates. Females were significantly larger than males: mean ± s.e. head widths were 0.61 ± 0.001 mm and 0.58 ± 0.002 mm for females and males respectively ($F_{1,622} = 410.3$, $p < 0.0001$), and their respective hind tibial lengths were 0.41 ± 0.001 mm and 0.39 ± 0.001 mm ($F_{1,622} = 52.3$, $p < 0.0001$). No trend between emergence position and head width or hind tibial length was apparent except that the last 1–5 wasps to emerge from an egg mass tended to have head widths and hind tibial lengths up to 0.05 mm smaller than previously emerged wasps.

Fecundity increased significantly with increases in female head width and hind tibial length (Fig. 3). However, regressions of fecundity against each of the two size measurements fitted poorly ($r^2 \leq 0.10$).

Sexual receptivity—The age of males had no significant effect on the number of pre-mating contacts, mating time or the num-

Table 4. Effect of virgin male age on their propensity to mate within 20 minutes of exposure to a virgin female (expressed as number of females mated). Also given is the number of females inseminated, number of pre-mating contacts, pre-mating time and mating time (last three values are mean ± s.e.). The number of males used to calculate each mean and s.e. is the number of males mated in 20 minutes (first row) from the 15 replicates.

	Male age (days)				
	1	5	10	15	20
No. mated	15a	15a	15a	15a	15a
No. inseminated	12a	12a	15a	12a	12a
No. contacts	2.6 ± 0.3a	1.9 ± 0.3a	1.8 ± 0.3a	2.7 ± 0.4a	2.2 ± 0.2a
Pre-mating time (s)	173 ± 37a	92 ± 17b	88 ± 21b	181 ± 33a	176 ± 24a
Mating time (s)	12.0 ± 1.3a	10.8 ± 1.0a	12.6 ± 0.6a	12.4 ± 0.8a	14.5 ± 2.1a

Row means followed by the same letter are not significantly different (G-test for first 2 rows, 1-way ANOVA for last 3 rows, $P > 0.05$).

Table 5. Effect of virgin female age on their propensity to mate within 20 minutes of exposure to a virgin male (expressed as number of females mated). Also given is the number of mated females that was successfully inseminated, number of pre-mating contacts, pre-mating time and mating time (last three values are mean ± s.e.). The number of females used to calculate each mean and s.e. is the number of females that mated within 20 minutes (first row) from the 15 replicates.

	Virgin female age (days)				
	1	5	10	15	20
No. mated	15a	15a	14ab	11b	10b
No. inseminated	12a	11a	10a	8ab	3b
No. contacts	2.6 ± 0.3a	5.3 ± 0.8b	8.6 ± 1.9b	5.0 ± 1.1b	5.8 ± 0.8b
Pre-mating time (s)	173 ± 37a	303 ± 43b	419 ± 113b	275 ± 93ab	370 ± 73b
Mating time (s)	12.0 ± 1.3a	11.5 ± 0.7a	8.1 ± 0.8b	10.9 ± 0.7a	9.3 ± 1.5ab

Row means followed by the same letter are not significantly different (G-test for first 2 rows, 1-way ANOVA for last 3 rows, $P > 0.05$).

ber of females mated or inseminated (Table 4). Pre-mating time was affected by male age with males aged 5 and 10 days old having a significantly shorter pre-mating time than males aged 1, 15 and 20 days old. No differences in male pre-mating or mating behaviour were observed between males of different ages.

In contrast, virgin female pre-mating and mating behaviours were affected by their age (Table 5). Females aged 5–20 days old tended to resist the males' mating attempts by moving away from them, aggressively chasing males away and/or refusing to allow males to copulate after mounting. The numbers of pre-mating contacts and pre-mating times were greater for females aged 5–20 days than for 1 day old females (Table 5). Females aged 1, 5 and 15 days old mated for significantly longer than 10 day old females. The number of females mated within 20 minutes and the number successfully inseminated decreased significantly with female age (Table 5).

The age of mated females also affected their pre-mating and mating behaviours, with mated females aged 5–20 days generally resisting mating attempts in the way described above for virgin females of different age. The mating propensity of mated 5–20 day old females was significantly less than that of one day old mated females (Table 6). The numbers of pre-mating contacts and pre-mating times increased significantly with female age, but mating time was not significantly affected by their age (Table 6).

Two-way ANOVA examining the effects of female age and sexual status (vir-

Table 6. Effect of age of previously-mated females on their propensity to mate within 20 minutes of exposure to a virgin male (expressed as number of females mated). Also given is the number of pre-mating contacts, pre-mating time and mating time (values are mean ± s.e.). The number of females used to calculate each mean and s.e. is the number of females that mated within 20 minutes (first row) from the 15 replicates.

	Age of previously-mated female (days)				
	1	5	10	15	20
No. mated	15a	6b	8b	9b	6b
No. contacts	4.7 ± 1.0a	9.7 ± 2.9b	7.0 ± 1.0ab	8.1 ± 1.1b	9.3 ± 3.4b
Pre-mating time (s)	235 ± 48a	549 ± 143b	373 ± 74ab	514 ± 97b	547 ± 205ab
Mating time (s)	8.6 ± 0.7a	8.4 ± 1.1a	6.1 ± 1.1a	7.4 ± 1.5a	9.7 ± 2.2a

Row means followed by the same letter are not significantly different (G-test for first row, 1-way ANOVA for last 3 rows, $P > 0.05$).

Table 7. Summary of results from 2-way ANOVA (log (x + 0.5) transformed) testing whether the individual and interactional effects of the factors, female age and mating status (virgin or once-mated), affected the number of pre-mating contacts, pre-mating time and mating time.

Factor	No. contacts	Pre-mating time	Mating time
Age	$F_{4,99} = 7.06$, $P < 0.0001$	$F_{4,99} = 4.50$, $P = 0.002$	$F_{4,99} = 3.31$, $p = 0.01$
Mating status	$F_{1,99} = 8.37$, $P = 0.005$	$F_{1,99} = 8.65$, $P = 0.004$	$F_{1,99} = 10.01$, $P = 0.002$
Age × mating status	$F_{4,99} = 0.89$, $P = 0.47$	$F_{4,99} = 0.83$, $P = 0.51$	$F_{4,99} = 0.96$, $P = 0.44$

gin or once-mated) on mating propensity indicated that the interaction between female age and mating status was not significant for all three measures of mating propensity (Table 7). As single factors, female age and mating status significantly affected the number of pre-mating contacts, pre-mating time and mating time (Table 7).

A log-linear analysis on the effect of female age on the propensity of virgin and mated females to mate within 20 minutes revealed that the model could be described best by two interactions: female mating status and the number of females mated, and female age and the number of females mated (Maximum likelihood $\chi^2 = 8.38$, df = 8, p = 0.40). The propensity of females to mate within 20 minutes was significantly greater for virgin females and younger females.

DISCUSSION

The results from this study suggest that strict local mating does not occur in *T. basalis* and that outbreeding away from the natal site may commonly occur. Results that are inconsistent with LMC theory include: (1) males do not achieve the maximum rate of insemination expected despite showing no apparent signs of sperm depletion, (2) males remain sexually receptive probably throughout their lifetime (Table 4), and (3) females can be mated multiple times, despite becoming decreasingly sexually receptive after mating and with age (Tables 6, 7). We discuss the implications that these results have on the mating system of *T. basalis* and ask whether this species is likely to outbreed.

Emergence of *T. basalis* females is concentrated during the early morning hours over several days (Wilson 1961; Fig. 1). In the field a dominant male usually guards the parasitised egg mass from which females are emerging. These females are his sisters unless the egg mass has been superparasitised. In the laboratory, the single male left on the mass successfully mated many females in succession, with sometimes up to 50 or more females emerging over several hours (Table 3). Females in all positions in the emergence sequence apparently received similar quantities of sperm because their offspring sex ratios were not affected by emergence position (Table 2; Fig. 2), even if those females all emerged and were mated on the same day by a single male (Table 3).

Although the above results suggest that a single *T. basalis* male can fully inseminate each of his female siblings from the same egg mass, two observations indicate that the dominant male does not achieve the maximum rate of insemination that is possible. First, only *ca* 80% of females were inseminated by dominant males (Table 1, see also Wilson 1961). Wilson (1961) proposed that temporary sperm depletion in the dominant male may be the cause. But even when large numbers of females emerged in a day, the offspring sex ratio produced by inseminated females did not vary with their position in the mating sequence (Table 3). In addition, uninseminated females appeared throughout the emergence sequence (Tables 1–3). Field observations have shown that a similar percentage of emerging females is not

mated by the dominant male guarding the egg mass (A.D. Loch and G.H. Walter unpublished data). Also, *ca* 18% of matings between virgin males and females in the laboratory do not lead to successful insemination (A.D. Loch and G.H. Walter unpublished data). In our experiments, we did not observe females to confirm they mated or to ascertain why they may not have mated. However, the high rate of uninseminated females is likely to be partly the consequence of simultaneous female emergences, during which males become occupied with some emerging females, while others move unmated to the top of the vial, a behaviour that has parallels in the field (A.D. Loch and G.H. Walter unpublished data).

The second observation suggesting that maximum insemination rates are not achieved by the dominant male, is that even those females that were inseminated produced proportionately more male offspring with age (Fig. 2). This trend has also been reported in other studies of *T. basalis* fecundity (Powell and Shepard 1982; Corrêa-Ferreira and Zamataro 1989; Awan et al. 1990) and in work on the confamilial *Telenomus busseolae* (Gahan) (Chabi Olaye et al. 1997). Females apparently do not receive sufficient sperm to fertilise all their eggs. However, temporary sperm depletion in males or insufficient sperm transfer by males are unlikely explanations. First, this trend was uniform for females in all emergence positions (Fig. 2), indicating that sperm depletion in the male was not the cause. Second, females held with males throughout their lifetime, and therefore assumed to be mated multiple times, also produce proportionately more male offspring with time (Powell and Shepard 1982; Awan et al. 1990), suggesting that this trend occurs irrespective of the number of times a female is mated. The reason for this trend is not clear, but a decrease in sperm viability over time is possible, or it could have a behavioural or physiological basis.

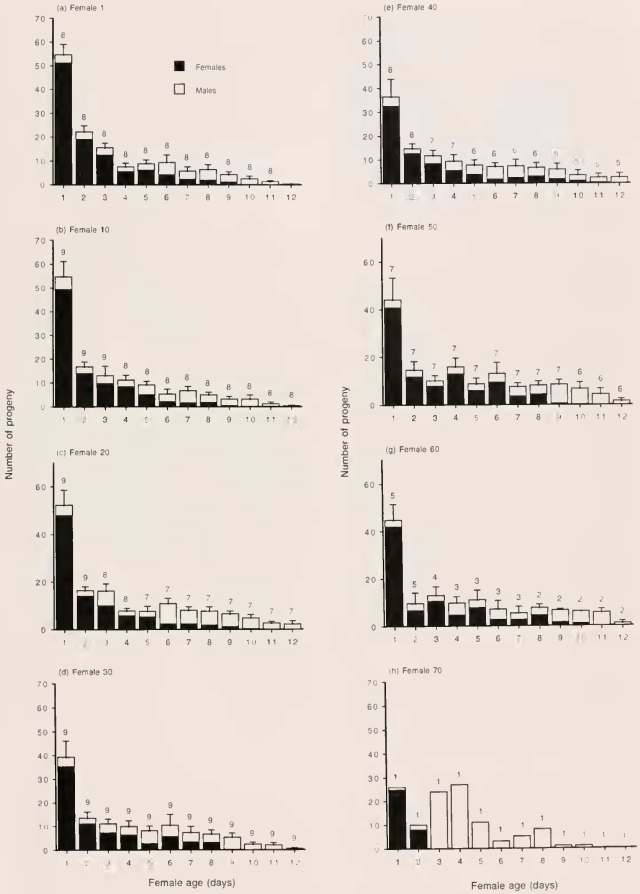
Results from this study suggest that *T. basalis* males do not become sperm depleted at rates of mating that are typical for this species in nature. In this study, green vegetable bug egg masses of 85 ± 5 eggs were used, and represent the largest known host masses for *T. basalis*, in terms of the number of eggs. The test males were, therefore, exposed to a high number and frequency of matings. In any case, dominant males in control of egg masses in the field are unlikely to become sperm depleted because changeovers in male dominance occur frequently (A.D. Loch and G.H. Walter unpublished data), and female emergence continues over several days (Fig. 1).

Sexual receptivity of *T. basalis* males appears unaffected by age (Table 4) and mating status, thus enabling males to mate probably throughout their lifetime. The only aspect of male sexual receptivity that was affected by age was pre-mating time, which was significantly shorter for 5–10 day old males than for 1, 15 and 20 day old males. They may be more receptive at 5–10 days because they emerge up to several days before females (Anon. 1939; Noble 1937; Smith 1945; Wilson 1961; Thomas 1972) and would therefore not normally need to mate immediately upon emergence.

In contrast, female sexual receptivity decreased rapidly after mating and with age (Tables 5–7). Such decreases are con-

→

Fig. 2. Number (mean ± s.e.) of progeny produced each day after emergence by inseminated *Trissolcus basalis* females. Data are presented separately for each group of parent females according to their position in the emergence sequence (i.e. (a) 1st, (b) 10th, (c) 20th, (d) 30th, (e) 40th, (f) 50th, (g) 60th and (h) 70th), and thus the sequence in which they were inseminated by the dominant male on their host egg mass. Numbers above error bars indicate the number of females still alive at that time.


(a) Female 1
Females
Males
(b) Female 10
(c) Female 20
(d) Female 30
(e) Female 40
(f) Female 50
(g) Female 60
(h) Female 70
Number of progeny
Number of progeny
Female age (days)
Female age (days)

sistent with LMC theory because mating is assumed to occur only at the natal site among siblings (Hamilton 1967). However, females can be mated multiple times, which is inconsistent with LMC theory. This inconsistency coupled with others such as male dispersal from the natal site (A. D. Loch and G. H. Walter unpublished data), and males remaining sexually receptive probably throughout their lifetime (Table 4) suggest that *T. basalis* of both sexes may mate away from the natal site and therefore outbreed.

Trissolcus basalis may outbreed if males and females can locate and/or attract each other once they have left the natal site. Males may be able to locate unrelated, newly-emerged (and thus sexually receptive) females directly, or they could do so indirectly by locating hosts parasitised by *T. basalis* and then competing with emerging males for mating access to females. Similarly, newly-emerged females may be able to locate males directly, or indirectly by searching for parasitised hosts with males in occupation. Currently, no evidence is available on whether males or females can locate potential mating partners away from the natal site. LMC models assuming strict local mating (Hamilton 1967) suggest that *T. basalis* males and females will not be able to locate each other in the field. However, in other hymenopterous species with female-biased sex ratios, such as *Spalangia cameroni* (Perkins) (Myint and Walter 1990) and *Pachycrepoideus vindemiae* (Rondani) (Nadel and Luck 1992), males are able to locate hosts and thus potential mating partners, a feature likely to be found in other species (Hardy 1994).

Males may not only be able to outbreed with newly-emerged virgin females but also with newly-emerged mated females because a previous mating encounter did not preclude females from mating again (Table 6). This result is not likely to be an artefact of laboratory conditions or procedures because females have been observed to be mated multiple times and by multiple males in the field (A. D. Loch and G. H. Walter unpublished data). Whether *T. basalis* females are truly polyandrous has yet to be established, for matings after the first successful mating may not lead to successful insemination. For instance, mating plugs may be used by males to ensure additional matings do not result in insemination.

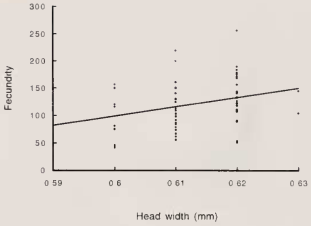


Fig. 3. Fecundity of *Trissolcus basalis* females in relation to head width ($y = 1711x - 928$, $r^2 = 0.10$, $n = 65$, $p = 0.01$). The trend for fecundity versus hind tibial length ($y = 1294x - 407$, $r^2 = 0.07$, $n = 65$, $p = 0.04$) is not shown because it was similar to the displayed trend. Both trends were determined irrespective of female position in the emergence sequence (see Table 2).

This study also made a number of findings pertaining to the fecundity of *T. basalis*. The mean fecundity recorded in this study is higher than fecundities recorded by Noble (1937), Ganesalingam (1966) and Thomas (1972), but similar to values recorded by Powell and Shepard (1982) and Corrêa-Ferreira and Moscardi (1994), and lower than fecundities recorded by Corrêa-Ferreira and Zamataro (1989) and Awan et al. (1990) for the same species. These differences are likely to be the result of differences in laboratory procedures and conditions, although differences in adult female size may have contributed because fecundity is greater for larger females (Fig. 3). The trend whereby fecundity peaked on the first day after female emergence and decreased rapidly over

time, differs somewhat from the results of Ganesalingam (1966) and Powell and Shepard (1982), who showed that fecundity peaked on day 2. These differences are less readily attributable to different laboratory procedures and conditions, and their significance is unclear. The claim by Field et al. (1998) that *T. basalis* is a synovigenic species was supported by our results because females laid eggs for 10–12 days with progressively fewer eggs each day (Fig. 2) despite sufficient hosts being available during the first few days for them to have deposited their lifetime complement of eggs then.

In conclusion, although uninseminated females leaving the egg mass may well be mated by the other males (also likely to be their siblings) that wait around the egg mass, the possibility that these females could mate unrelated males near or away from the natal site may not be low. Although we have no direct evidence of *T. basalis* outbreeding in nature, the results from this study and other related studies (A.D. Loch and G.H. Walter unpublished data), suggest that it may be more frequent than anticipated by LMC theory. In addition, outbreeding is likely to occur when > 1 female oviposits in an egg mass. Further research investigating the mating system of *T. basalis* is required before the question of the species' outbreeding can be resolved. Specific issues that need to be addressed include the questions of whether *T. basalis* haa a means of mate-attraction, and whether *T. basalis* females are truly polyandrous.

ACKNOWLEDGMENTS

We thank Tony Clarke for discussing aspects of this manuscript, and Norman Johnson (Ohio State University) for confirming the identification of *Trissolcus basalis*. We also thank Ian Hardy and Peter Mayhew for their comments and suggestions on earlier versions of the manuscript. The senior author was supported by a Grains Research and Development Corporation Junior Research Fellowship.

LITERATURE CITED

Anon. 1939. The egg parasite of the green vegetable bug. *The Agricultural Gazette of New South Wales* 50: 277–278.

Awan, M. S., Wilson, L. T. and Hoffman, M. P. 1990. Comparative biology of three geographic populations of *Trissolcus basalis* (Hymenoptera: Scelionidae). *Environmental Entomology* 19: 387–392.

Chabi Olaye, A., Schulthess, F., Shanower, T. G. and Bosque-Pérez, N. A. 1997. Factors influencing the developmental rates and reproductive potentials of *Telenomus busseolae* (Gahan) (Hym.: Scelionidae), an egg parasitoid of *Sesamia calamistis* Hampson (Lep.: Noctuidae). *Biological Control* 8: 15–21.

Corrêa-Ferreira, B. S. and Moscardi, F. 1994. Temperature effect on the biology and reproductive performance of the egg parasitoid *Trissolcus basalis* (Woll.). *Anais da Sociedade Entomologica do Brasil* 23: 399–408.

Corrêa-Ferreira, B. S. and Zamataro, C. E. O. 1989. Reproductive capability and longevity of the egg parasitoids *Trissolcus basalis* (Wollaston) and *Trissolcus mitsukurii* Ashmead (Hymenoptera: Scelionidae). *Revista Brasileira de Biologia* 49: 621–626.

Field, S. A., Keller, M. A. and Austin, A. D. 1998. Field ecology and behaviour of the egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae). *Transactions of the Royal Society of South Australia* 122: 65–71.

Ganesalingam, V. K. 1966. Some environmental factors influencing parasitisation of the eggs of *Nezara viridula* L. (Pentatomidae) by *Telenomus basalis* Wollaston (Hymenoptera: Scelionidae). *Ceylon Journal of Science (Biological Sciences)* 6: 1–14.

Godfray, H. C. J. 1994. *Parasitoids: behavioural and evolutionary ecology*. Princeton University Press, Princeton, 473 pp.

Hamilton, W. D. 1967. Extraordinary sex ratios. *Science* 156: 477–488.

Hardy, I. C. W. 1994. Sex ratio and mating structure in the parasitoid Hymenoptera. *Oikos* 69: 3–20.

Hardy, I. C. W., Dijkstra, L. J., Gillis, J. E. M. and Luft, P. A. 1998. Patterns of sex ratio, virginity and developmental mortality in gregarious parasitoids. *Biological Journal of the Linnean Society* 64: 239–270.

Hardy, I. C. W., Ode, P. J. and Strand, M. R. 1993. Factors influencing brood sex ratios in polyembryonic Hymenoptera. *Oecologia* 93: 343–348.

Hardy, I. C. W., Pedersen, J. B., Sejr, M. K. and Linderoth, U. H. 1999. Local mating, dispersal and sex ratio in a gregarious parasitoid wasp. *Ethology* 105: 57–72.

Kelly, G. L. 1987. Factors affecting the success of *Trissolcus basalis* (Hymenoptera: Scelionidae) as a biological control agent of the green vegetable bug,

Nezara viridula (Hemiptera: Pentatomidae). Unpublished PhD thesis, University of Sydney, Sydney, 329 pp.

Molbo, D. and Parker Jr, E. D. 1996. Mating structure and sex ratio variation in a natural population of *Nasonia vitripennis*. *Proceedings of the Royal Society of London. Series B. Biological Sciences* 263: 1703–1709.

Myint, W. W. and Walter, G. H. 1990. Behaviour of *Spalangia cameroni* males and sex ratio theory. *Oikos* 59: 163–174.

Nadel, H. and Luck, R. F. 1985. Span of female emergence and male sperm depletion in the female-biased, quasi-gregarious parasitoid, *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae). *Annals of the Entomological Society of America* 78: 410–414.

Nadel, H. and Luck, R. F. 1992. Dispersal and mating structure of a parasitoid with a female-biased sex ratio: implications for theory. *Evolutionary Ecology* 6: 270–278.

Noble, N. S. 1937. An egg parasite of the green vegetable bug. *Agricultural Gazette of New South Wales* 48: 337–341.

Ode, P. J., Antolin, M. F. and Strand, M. R. 1997. Constrained oviposition and female-biased sex allocation in a parasitic wasp. *Oecologia* 109: 547–555.

Powell, J. E. and Shepard, M. 1982. Biology of Australian and United States strains of *Trissolcus basalis*, a parasitoid of the green vegetable bug, *Nezara viridula*. *Australian Journal of Ecology* 7: 181–186.

Smith, J. H. 1945. Useful parasitic insects. *Queensland Agricultural Journal* 60: 340–351.

Thomas, J. W. Jr. 1972. Evaluation of *Trissolcus basalis* (Wollaston) as an egg parasite of *Nezara viridula* (Linnaeus). Unpublished MSc thesis, Louisiana State University, Louisiana, 99 pp.

van den Assem, J., Gijswijt, M. J. and Nübel, B. K. 1980. Observations on courtship - and mating strategies in a few species of parasitic wasps (Chalcidoidea). *Netherlands Journal of Zoology* 30: 208–227.

Waage, J. K. and Lane, J. A. 1984. The reproductive strategy of a parasitic wasp. II. Sex allocation and local mate competition in *Trichogramma evanescens*. *Journal of Animal Ecology* 53: 417–426.

Waage, J. K. and Ng, S. M. 1984. The reproductive strategy of a parasitic wasp. I. Optimal progeny and sex allocation in *Trichogramma evanescens*. *Journal of Animal Ecology* 53: 401–415.

Walter, G. H. and Clarke, A. R. 1992. Unisexual broods and sex ratios in a polyembryonic encyrtid parasitoid (*Copidosoma* sp.: Hymenoptera). *Oecologia* 89: 147–149.

Wilson, F. 1961. Adult reproductive behaviour in *Asolcus basalis* (Hymenoptera: Scelionidae). *Australian Journal of Zoology* 9: 739–751.